

Having described the invention, what is claimed is:

1. A method for regulating the abnormal lymphocytic cell cycle of continuously dividing T and B lymphocyte blood cells of a mammal comprising the steps of: obtaining a cell sample of normal lymphocyte blood cells from the mammal; preparing a lysate from the cell sample; and administering the lysate to the mammal to assist in regulating the mammal's lymphocytic cell cycle.
2. A method according to Claim 1, further comprising the step of determining an initial status of the lymphocytic cell cycle of the mammal.
3. A method according to Claim 2, further comprising the step of: determining a later status of the lymphocytic cell cycle after administering the lysate to the mammal and comparing the initial status of the mammal's lymphocytic cell cycle to the later status of the mammal's lymphocytic cell cycle to measure any effect of the lysate.
4. A method according to Claim 3, further comprising the step of: administering additional lysate to the mammal to further regulate the mammal's lymphocytic cell cycle.
5. A method according to Claim 2, wherein the step of determining the initial status of the lymphocytic cell cycle of the mammal comprises the steps of: adding lysing buffer to a portion of the cell sample; adding DNA stain and RNase to the portion of the cell sample; analyzing the portion of the DNA stained cell sample with flow cytometry to determine the DNA distribution in the lymphocytic cell cycle.

6. A method according to Claim 5, wherein the step of analyzing the portion of the cell sample further comprises the step of: passing the DNA stained portion of the cell sample through a fluorescence spectrometer.

7. A method according to Claim 6, wherein the step of determining the initial status of the lymphocytic cell cycle of the mammal further comprises the step of: calculating the DNA distribution in the lymphocytic cell cycle by parametric analysis of the accumulated fluorescence data to produce a DNA histogram.

8. A method according to Claim 1, wherein the step of obtaining a cell sample from the mammal comprises: collecting a blood sample; separating the erythrocytes from the blood sample; separating the T lymphocytes from separated erythrocytes; and suspending the T lymphocytes in normal saline.

9. A method according to Claim 8, wherein the step of collecting a blood sample comprises: venipuncture in heparinized tubes.

10. A method according to Claim 8, wherein the step of preparing a lysate from the cell sample of the mammal further comprises the steps of: isolating lymphocytes from the blood sample; propagating the isolated lymphocytes; and lysing the propagated lymphocytes.

11. A method according to Claim 10, wherein the step of isolating lymphocytes from the blood sample further comprises the steps of: separating erythrocytes from the blood sample by a Ficoll-Hypaque density-gradient technique; centrifuging the blood sample; separating at least one of the lymphocytic layers from the centrifuged erythrocytes; and washing the at least one separated lymphocytic layer with normal saline by centrifugation.

12. A method according to Claim 11, wherein the step of separating at least one of the lymphocytic layers from the centrifuged erythrocytes comprises separating the mixed lymphocyte layers from the centrifuged erythrocytes.

13. A method according to Claim 11, wherein the step of separating at least one of the lymphocytic layers from the centrifuged erythrocytes comprises separating the lymphocyte layers from the centrifuged erythrocytes.

14. A method according to Claim 13, wherein the step of separating at least one of the lymphocytic layers from the centrifuged erythrocytes further comprises the step of isolating at least one specific subclass of T lymphocytes from other lymphocytes.

15. A method according to Claim 11, wherein the step of isolating the lymphocytes from the blood sample further comprises the step of: diluting the separated erythrocytes with phosphate buffered saline prior to overlaying the separated erythrocytes on isolymph.

16. A method according to Claim 10, wherein the step of propagating the lymphocytes further comprises the steps of: adding the isolated lymphocytic cells to a cell culture flask containing a cell growth medium and incubating at about 37°C.

17. A method according to Claim 16, wherein the cell growth medium comprises RPMI 1640 medium supplemented with bovine calf serum.

18. A method according to Claim 16, wherein the step of propagating the lymphocytes further comprises the steps of: centrifuging the incubated lymphocytes; removing the supernate; and washing the lymphocytes in normal saline by centrifugation.

19. A method according to Claim 10, wherein the step of separating lysate from the propagated lymphocytes further comprises the steps of: suspending the propagated lymphocytes in normal saline solution; sonicating the suspended lymphocytes; and filtering the sonicated cells to sterilize the lysate.

20. A method according to Claim 1, wherein the step of administering the lysate to the mammal comprises the steps of: diluting the lysate obtained from the cell sample to obtain a desired lysate concentration, and injecting the desired dose subcutaneously.

21. A method according to Claim 19, wherein the step of administering the lysate to the mammal further comprises the step of determining the desired lysate concentration by skin testing.

22. A method for regulating an abnormal lymphocytic cell cycle of a mammal comprising the steps of: obtaining an initial blood sample from the mammal; determining an initial status of the lymphocytic cell cycle of the mammal; administering a treatment of ALF to the mammal; obtaining a second blood sample from the mammal after administering the ALF; and determining a post-treatment status of the lymphocytic cell cycle, whereby the regulatory effect of the ALF treatment on the lymphocytic cell cycle is determined by comparing the initial status of the lymphocytic cell cycle and the post-treatment status of the lymphocytic cell cycle.

23. A method according to Claim 22, further comprising the step of: administering additional ALF to the mammal to further study the effect of the ALF treatment on the mammal's lymphocytic cell cycle.

24. A method according to Claim 22, wherein the step of determining the initial status of the lymphocytic cell cycle of the mammal comprises the steps of: adding lysing buffer to a portion of the blood sample; adding DNA stain and RNase to the portion of the cell sample; analyzing the portion of the DNA stained cell sample with flow cytometry to determine the DNA distribution in the lymphocytic cell cycle.

25. A method according to Claim 24, wherein the step of analyzing the portion of the cell sample further comprises the step of: passing the DNA stained portion of the cell sample through a fluorescence spectrometer.

26. A method according to Claim 25, wherein the step of determining the initial status of the lymphocytic cell cycle of the mammal further comprises the step of: calculating the DNA distribution in the lymphocytic cell cycle by parametric analysis of the accumulated fluorescence data to produce a DNA histogram.

27. A method according to Claim 22, wherein the step of collecting a blood sample comprises: venipuncture in heparinized tubes.

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28. A method for regulating the abnormal lymphocytic cell cycle of continuously dividing lymphocytic blood cells of a mammal comprising the steps of: obtaining an initial blood sample from the mammal; determining an initial blood status of the lymphocytic cell cycle of the mammal; preparing a lysate from the normal lymphocytes of the blood sample, administering a treatment of the lysate to the mammal; obtaining a second blood sample from the mammal after administering the lysate; and determining a post-treatment status of the lymphocytic cell cycle, whereby the regulatory effect of the treatment is determined by comparing the initial status of the lymphocytic cell cycle and the post-treatment status of the lymphocytic cell cycle, wherein the step of preparing the lysate from the blood sample further comprises the steps of: isolating lymphocytes from the blood sample; propagating the isolated lymphocytes; and preparing a lysate from the propagated lymphocytes.

29. A method according to Claim 28, wherein the step of isolating lymphocytes from the blood sample further comprises the steps of: separating erythrocytes from the blood sample by a Ficoll-Hypaque density-gradient technique; centrifuging the erythrocytes; separating at least one of the lymphocytic layers from the centrifuged erythrocytes; and washing the separated lymphocytic layer with normal saline by centrifugation.

30. A method according to Claim 29, wherein the step of separating at least one of the lymphocytic layers from the centrifuged erythrocytes comprises separating the mixed lymphocyte layers from the centrifuged erythrocytes.

31. A method according to Claim 29, wherein the step of separating at least one of the lymphocytic layers from the centrifuged erythrocytes comprises separating the lymphocyte layers from the centrifuged erythrocytes.

32. A method according to Claim 31, wherein the step of separating at least one of the lymphocytic layers from the centrifuged erythrocytes further comprises the step of isolating at least one specific subclass of T lymphocytes from other lymphocytes.

33. A method according to Claim 29, wherein the step of isolating lymphocytes from the blood sample further comprises the step of: diluting the separated erythrocytes with phosphate buffered saline prior to overlaying the separated erythrocytes on isolymph.

34. A method according to Claim 28, wherein the step of propagating the lymphocytes further comprises the steps of: adding the isolated lymphocytic cells to a cell culture flask containing a cell growth medium and incubating at about 37°C.

35. A method according to Claim 34, wherein the cell growth medium comprises RPMI 1640 medium.

36. A method according to Claim 34, wherein the step of propagating the lymphocytes further comprises the steps of: centrifuging the incubated lymphocytes; removing the supernate; and washing the lymphocytes in normal saline by centrifugation.

37. A method according to Claim 28, wherein the step of preparing a lysate from the propagated lymphocytes further comprises the steps of: suspending the propagated lymphocytes in normal saline solution; sonicating the suspended lymphocytes; and filtering the sonicated cells to sterilize the lysate.

38. A method according to Claim 28, wherein the step of administering the lysate to the mammal comprises the steps of: diluting the lysate obtained from the blood sample to obtain a desired lysate concentration, and injecting subcutaneously a dose of the diluted lysate.

39. A method according to Claim <sup>38</sup>~~41~~, wherein the step of administering the lysate to the mammal further comprises the step of predetermining the desired lysate concentration by skin testing.

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40. A method of treating a mammalian individual having abnormal T and B lymphocyte parameters, the method comprising the steps of:

- (a) collecting an initial blood sample from the individual;
- (b) isolating at least one type or subset of normal lymphocytes from the initial blood sample;
- (c) propagating the isolated normal lymphocytes;
- (d) lysing the propagated lymphocytes;
- (e) determining a therapeutic dose of the lysate for the individual; and
- (f) administering at least one therapeutic dose of the lysate to the individual.

41. The method of Claim 40, further comprising the steps of: initially determining T and B lymphocyte parameters of the individual, and then, after administering the at least one therapeutic dose of the lysate to the individual, determining T and B lymphocyte parameters of the individual.

42. The method according to Claim 41, wherein the steps of determining the T and B lymphocyte parameters of the individual comprises: determining the lymphocytic cell cycle of the individual.

43. The method according to Claim 42, wherein the step of determining the lymphocytic cell cycle of the individual further comprises the steps of: adding lysing buffer to a portion of the blood sample; adding DNA stain and RNase to the portion of the cell sample; analyzing the portion of the DNA stained cell sample with flow cytometry to determine the DNA distribution in the lymphocytic cell cycle.

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44. The method according to Claim 43, wherein the step of analyzing the portion of the cell sample further comprises the step of: passing the DNA stained portion of the cell sample through a fluorescence spectrometer.

45. The method according to Claim 44, wherein the step of determining the initial status of the lymphocytic cell cycle further comprises the step of: calculating the DNA distribution in the lymphocytic cell cycle by parametric analysis of the accumulated fluorescence data to produce a DNA histogram.

46. The method according to Claim 41, wherein the steps of determining the T and B lymphocyte parameters of the individual comprise: determining T and B lymphocyte and subset numbers.

47. The method of Claim 40, further comprising the steps of: determining the initial cell mediated immunity of the individual, and then determining the cell mediated immunity of the individual after administering the at least one therapeutic dose of the lysate.

48. The method of Claim 40, further comprising the steps of: measuring the clinical symptoms and signs of the individual, and then determining the symptoms and signs of the individual after administering the at least one therapeutic dose of the lysate.

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